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Sclerotinia Twig Blight on Trees and Cottony Rot on Fruits of Satsuma Mandarin Caused by *Sclerotinia sclerotiorum*

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Sclerotinia twig blight on trees and cottony rot on fruits of Satsuma mandarin were observed in Cheju, Korea in 1996 and 1997. The causal fungus causing citrus twig blight and cottony rot was identified as *Sclerotinia sclerotiorum* which had cup-shaped apothecia bearing hyaline and clavate asci and periphyses on sclerotium. Symptoms were produced on twigs, developing young leaves, fully developed new leaves, and fruits 2-9 days after wound inoculation, but only on twigs with young lateral buds and developing young leaves by unwound inoculation. The fungus grew well between 10 and 27°C, but optimum temperature was 24°C on potato dextrose agar. Most varieties were highly susceptible to *S. sclerotiorum*, whereas some varieties such as Ichinan were relatively resistant among twelve Satsuma mandarin varieties in the field. This is the first record of Sclerotinia twig blight on trees and cottony rot on stored fruits of Satsuma mandarin in Korea.

Keywords : cottony rot, Satsuma mandarin, *Sclerotinia sclerotiorum*, Sclerotinia twig blight.

Sclerotinia sclerotiorum (Lib.) de Bary occurs on a wide range of hosts, including many fruits, vegetables, and flowers (Abawi and Grogan, 1979; Farr et al., 1990; Purdy, 1979). The fungus also causes Sclerotinia twig blight on citrus trees and cottony rot on stored fruits. The latter caused substantial losses of lemons during storage in the coastal areas of California, but it has not been generally a major problem on citrus (Whiteside et al., 1988). There has been no record of Sclerotinia twig blight on trees and cottony rot on stored fruits of Satsuma mandarin (*Citrus unshiu*) in Korea (The Korean Society of Plant pathology, 1998), but the diseases were sporadically observed on twigs, leaves and stored fruits of Satsuma mandarin in Cheju, Korea in 1996 and 1997. Especially about 35% of seedlings planted in a nursery of Satsuma mandarin orchard were severely damaged by Sclerotinia twig blight in Seoquipo, Cheju in 1997. These studies were conducted to investigate the occurrence of Sclerotinia twig blight disease and cottony rot disease on Satsuma mandarin, to determine their causal agent and to evaluate the resistance and susceptibility of several varieties of Satsuma mandarin to *S. sclerotiorum* in the field.

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Materials and Methods

Fungal isolates. Numerous fungal isolates were obtained from diseased twigs, leaves and fruits of Satsuma mandarin. A piece of tissue ca. 5 mm^2 was cut from the edge of the diseased area, treated with 95% ethyl alcohol for 1 min and rinsed with sterile water, followed by placing on potato dextrose agar (PDA). Induction of apothecial production was attempted by Onkar and James' method (1995). After numerous sclerotia were formed on PDA, 10 ml of sterilized water was added and sealed with parafilm. The plate was incubated under 12-hour-alternating NUV-light illumination at 15°C to induce apothecium. Morphological characteristics of sclerotia, apothecia, asci and ascospores were examined by light- and stereo-microscopy in order to identify the fungus.

Pathogenicity test. Three isolates were selected from apothecia isolated from each of diseased twigs (isolate S1), leaves (isolate S2) and fruits (isolate S3) of Satsuma mandarin, respectively. Twigs with young lateral buds less than 3 mm in length, twigs without young lateral buds, developing young leaves less than 6 cm in length, fully developed new leaves, 1-year-old leaves, and fruits were used to test their pathogenicity at different growth stages of Satsuma mandarin. The materials which were inoculated with a fungal suspension (4×10^4 conidia/ml) until run-off were wrapped with polyethylene film to give 100% relative humidity and kept at 25±2°C in a growth chamber. Wounding and non-wounding inoculations were made for each experiment with three replicates. Disease severity was rated 2-9 days after inoculation.

The relationship of temperature to fungal growth. The relationship of temperature to fungal growth was determined for three isolates used for the pathogenicity test. Each isolate was grown on three plates of PDA at 0, 5, 10, 15, 21, 24, 27, 30 and 33°C in the dark. Colony diameters were measured after 4 days of incubation.

Variety resistance. Twelve Satsuma mandarin varieties were evaluated for their resistance to Sclerotinia twig blight. Disease severity was measured on Kitakuchi, Ueno, Miyamoto, Okistu, Oura, Yamasita beni, Miyagawa, Iwasaki, Sichimara, Zabara, Yamagawa, and Ichinan, which were naturally infected by *S. sclerotiorum* in the experimental orchards, the Cheju Citrus Research Institute, Namwon, Cheju. Percentages of diseased trees and numbers of diseased twigs of ca. 4 cm long per tree were examined on August 20, 1997. Five to eight trees per variety in each of 3 plots were investigated in the field.

Results

Symptoms. Sclerotinia twig blight and cottony rot diseases were sporadically observed on twigs, leaves and stored fruits of Satsuma mandarin in Cheju, Korea in 1996 and 1997. Especially about 35% of seedlings were severely damaged by Sclerotinia twig blight in a nursery of Satsuma

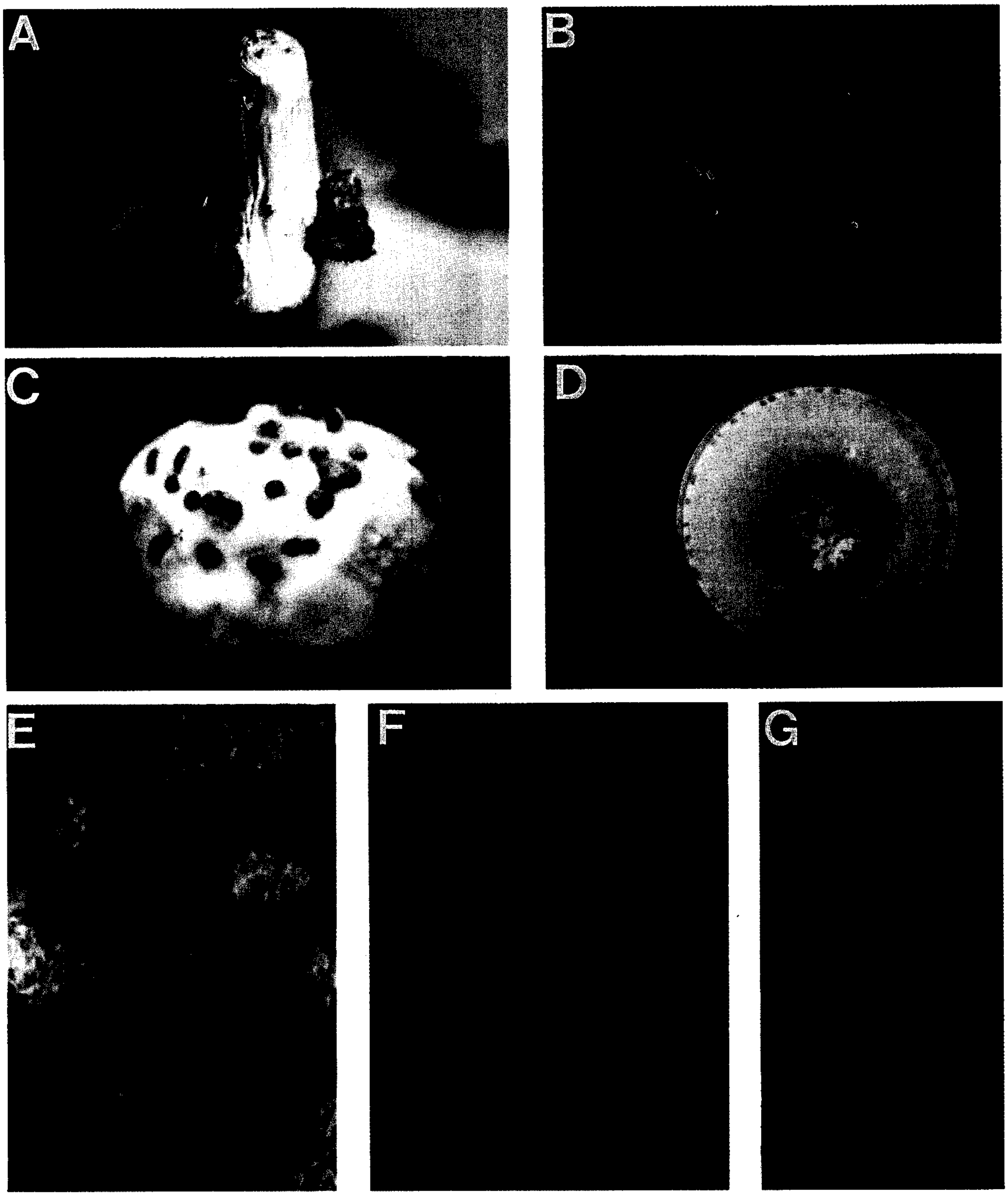

Fig. 1. Symptoms of Sclerotinia twig blight on twig (A) and leaf (B) and cottony rot on fruit (C) of Satsuma mandarin and morphology of *Sclerotinia sclerotiorum*. Colony on potato dextrose agar (D), apothecia (E) on sclerotia, asci (F) and ascospores (G) of *S. sclerotiorum*. Scale bars represent 50 μm

mandarin orchard, Seoquipo, Cheju in 1997. Symptoms on twigs appeared as brown water-soaked lesions and white mycelia were grown on the lesions. Pale-brown gum was extruded from the opposite site of the lesions as the disease developed. Later, the bark turned greyish brown as the disease developed severely and became shredded into longitudinal fibrous strips. The color was dark brown in the margin of infected tissues and xylem turned pale-yellow to brown.

The leaves and fruits above the diseased parts wilted and 1-3 sclerotia ranged 2.2-8.1 mm (average 3.6 mm) were formed on the lesions (Fig. 1A). Brown water-soaked lesions developed rapidly on diseased leaves and dark sclerotia were formed on the lesions after white mycelia developed. The diseased leaves were wilted and fallen in a few days after appearance of the symptoms (Fig. 1B). Pale yellow lesions occurred on fruits during storage and white mycelia covered the surface of the fruits and numerous sclerotia were formed on the lesions (Fig. 1C).

Identification of the pathogen. *Sclerotinia* sp. was consistently isolated from the lesions on the diseased parts of Satsuma mandarin. The cultures were white on PDA and the growth rates at 24°C were 21±2 mm/day, but pale brown to dark grey mycelia were also observed in old cultures. Various kinds of sclerotia ranging 2.1-8.1 mm (average 3.6 mm) in diameter were formed on the margin of the plates (Fig. 1D). Many apothecia were produced 40-50 days after incubation of sclerotia in water by Onkar and James' method (1995). One to six apothecia with pale yellow to pale brown color developed on a sclerotium. Yellowish brown to light brown stalks without ring and volva, 3.5-5.5 mm (average 4.4 mm) tall, arose singly or in 2-6 small groups from a sclerotium (Fig. 1E). Apothecia were usually cup-shaped and yellowish brown, and their length was 0.7-3.7 mm (average 2.5 mm). Asci were long club-shaped, and some of them had paraphyses and were hyaline to pale yellow. The length of asci was 86.7-137.7 μm (average 123.5 μm), the width, 6.1-8.2 μm (average 7.3 μm) and the ratio of length to width, 15.2-20.4 (average 17.2) (Fig. 1F). Ascus contained 8 ascospores hyaline to pale yellow, non-septate and elliptical, containing 1-2 oil drops in both ends. Ascospores were 10.2-12.8 μm (average 11.2 μm) long and 5.1-6.6 μm (average 5.7 μm) wide and the ratio of length to width was 1.8-2.3 (average 2.0) (Fig. 1G) (Table 1). The morphological and cultural characteristics of the fungus from Satsuma mandarin were in accordance with those of *Sclerotinia sclerotiorum* (Lib.) de Bary (Ainsworth et al., 1973; Dennis, 1981). This is the first record of Sclerotinia twig blight disease on trees and cottony rot disease on stored fruits of Satsuma mandarin in Korea, which are commonly referred to 'Gyunhaekbyeong' in Korean.

Pathogenicity. The symptoms on twigs, leaves and fruits at different growth stages induced by artificial inoculation with three isolates were identical to those observed in the field and during storage, respectively, but there was no difference in pathogenicity among three isolates. The fungus was reisolated from lesions on inoculated twigs, leaves and

Table 1. Comparison of morphological characteristics of the present isolates with *Sclerotinia sclerotiorum* previously described

Characteristics		Present isolates	*Sclerotinia sclerotiorum*[a]
Sclerotia	Shape	sausage-like, globular or irregular, dark brown to black	initially cushion-like or short-cylindrical and white, finally black
	Size (mm)	2.2-8.1 (average 3.6)	below 12[b]
Apothecia			
Disc	Shape	cup-shaped with concave yellowish-brown	cup-shaped with concave yellowish-brown
	Size (mm)	0.7-3.7 (average 2.5)	up to 10
Stalk	Shape	arising singly or in small groups (2-6) from sclerotia, yellowish-brown to light brown, cylindrical, smooth	arising singly or in small groups from sclerotia, slender light-brown, smooth
	Size (mm)	3.5-5.5 (average 4.4)	–[c]
Asci	Shape	cylindric-clavate, hyaline. 8-spored, cylindrical paraphyses	cylindric-clavate, hyaline, 8-spored, cylindrical paraphyses
	Size (μm)	86.7-137.7×6.1-8.2 (average 123.5×7.3)	up to 130×10
	L/W	15.2-20.4 (average 17.2)	–[c]
Ascospores	Shape	hyaline, non-septate, elliptical, 1-2 oil drops	hyaline, non-septate, elliptical
	Size (μm)	10.2-12.8×5.1-6.6 (average 11.2×5.7)	9-13×4-6.5
	L/W	1.8-2.3 (average 2.0)	–[c]

[a]From Dennis, E. W. G. (1981).
[b]From Walter, R. E. (1978).
[c] Not described.

Table 2. Pathogenicity of 3 isolates of *Sclerotinia sclerotium* from twigs (S1), leaves (S2) and fruits (S3) of Satsuma mandarin

Parts of inoculation	Treatment	Isolate		
		S1	S2	S3
Twigs with young lateral buds	Wounded	+[a]	+	+
	Unwounded	+	+	+
	Control	–	–	–
Twigs without young lateral buds	Wounded	+	+	+
	Unwounded	–	–	–
	Control	–	–	–
Developing young leaves	Wounded	++	++	++
	Unwounded	+	+	+
	Control	–	–	–
Fully developed new leaves	Wounded	+	+	+
	Unwounded	–	–	–
	Control	–	–	–
One-year-old leaves	Wounded	–	–	–
	Unwounded	–	–	–
	Control	–	–	–
Fruits	Wounded	+	+	+
	Unwounded	–	–	–
	Control	–	–	–

[a]++: severe symptom developed from 2 days after inoculation, + : mild symptom developed 6-9 days after inoculation, –: no symptom developed until 30 days.

fruits. Symptoms of twig blight were developed on both wounded and unwounded twigs with young lateral buds 7 days after inoculation, but not developed on unwounded twigs without young lateral buds. Leaf blight symptoms were developed on wounded developing young leaves less than 6 cm in length 2 days after inoculation and on unwounded developing young leaves 7 days after inoculation, respectively. The leaf symptoms were developed only on wounded fully developed new leaves 7 days after inoculation, but no symptom was observed on both wounded and unwounded 1-year-old leaves by artificial inoculation. Symptoms of cottony rot on fruit were developed by wound inoculation on fruits 6 days after inoculation, but no symptom was observed on unwounded fruits even 30 days after inoculation (Table 2).

Mycelial growth. There was no significant difference among the growth patterns of three isolates at different temperatures. The fungus grew well between 10 and 27°C, but optimum temperature for the growth of *S. sclerotiorum* was 24°C on PDA. The fungus did not grow under 10°C and above 30°C (Fig. 2).

Variety resistance. Reactions of Satsuma mandarin to *S. sclerotium* varied with varieties in the field. Most varieties of Satsuma mandarin were highly susceptible to *S. sclerotiorum*, whereas some varieties such as Ichinan were relatively more resistant among twelve varieties investigated in the field (Table 3).

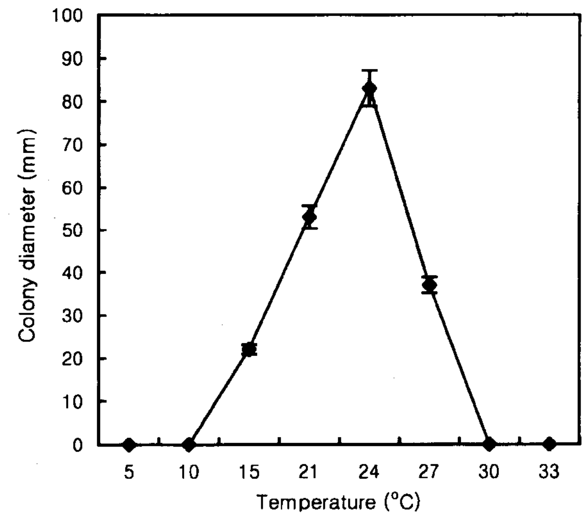

Fig. 2. Mycelial growth of *Sclerotinia sclerotiorum* on potato dextrose agar 4 days after incubation at different temperatures.

Table 3. Reactions of 12 varieties of Satsuma mandarin to *Sclerotinia sclerotium* in the naturally infested field, Cheju, Korea in 1997

Variety	Diseased trees (%)	Number of infected twigs per tree
Miyamoto	90.5 X[a]	3.9 X
Okistu	90.0 X	2.0 Y
Yamasita beni	90.0 X	1.4 Y
Ueno	85.0 X	4.0 X
Oura	85.0 X	1.6 Y
Iwasaki	84.2 XY	1.3 Y
Miyagawa	80.0 XY	1.4 Y
Kitakuchi	70.0 XY	5.0 X
Sichimara	70.0 XY	0.7 Y
Zabara	55.0 XYZ	0.6 Y
Yamagawa	44.4 YZ	0.4 Y
Ichinan	27.8 Z	0.2 Y

[a]Five to eight trees per variety in each of 3 plots were investigated in a field. Means followed by the same letter in each column are not significantly different (P=0.05) by Duncan's multiple range test.

Discussion

Sclerotinia sclerotiorum has a wide host range of 383 plant species in 225 genera, 64 families (Purdy, 1979). Generally vegetables such as crucifers, cucurbits, solanaceous plants are cultivated under the citrus trees in farmers' orchards in Cheju, Korea. These vegetables as well as weeds such as *Trifolium repens*, *Ixeris dentata*, and *Solanum nigrum* are the important hosts of *S. sclerotiorum* (Shin and Lee, 1987).

Although detailed epidemiological information is not available on Sclerotinia twig blight on trees and cottony rot on fruits of Satsuma mandarin, they might contribute to the development of epidemics as the primary source of inoculum of *S. sclerotiorum*. Therefore, sanitation of citrus orchards including eradication of weeds is very important for the prevention of Sclerotinia twig blight disease and cottony rot disease caused by *S. sclerotiorum* (Walter et al., 1978; Whiteside, 1988). Recently, cultivation of nonhost plants under citrus trees is considered as one of the proper cultural practices to prevent both soil invasion and introduction of the fungus into the orchards.

Infection of aboveground plant parts of citrus by *S. sclerotiorum* is initiated by ascosporic inoculum. Apothecia develop from sclerotia overwintered in and on soils under humid conditions at around 10°C (Abawi and Grogan, 1975). Ascospores, which develop within the apothecium, are disseminated by air currents and initiate infection when they contact susceptible tissues (Adams and Ayers, 1979). Since ascospores rarely penetrate healthy tissues directly, Satsuma mandarin generally is predisposed by wounds caused by mechanical injury and by sunscold or cold injury to Sclerotinia twig blight on trees and cottony rot on fruits (Walter et al., 1978). In this experiment, we also observed rapid development of lesions on wounded twigs, leaves and fruits. However, *S. sclerotiorum* infected directly the host tissues on unwounded twigs with young lateral buds and developing young leaves. This suggests that Sclerotinia twig blight on young shoots and new leaves can occur by direct penetration at early growing season in spring.

Invasion of healthy plant parts is made by mycelium derived from ascospores. After thorough invasion of host tissue by mycelium, abundant white mycelia develop when environmental conditions are favorable, and subsequently, sclerotia are produced externally on affected plant parts (Purdy, 1979). Optimum temperature for mycelial growth of *S. sclerotiorum* isolated in this experiment was 24°C on PDA, which was consistent to the previous results (Abawi and Grogan, 1975; Capellini, 1960; Shin and Lee, 1987). The constant temperature of 20-25°C is near optimum for ascospore germination and mycelial growth as well as lesion initiation and development of Sclerotinia diseases (Abawi and Grogan, 1975). Since the weather of Cheju island during flowering season of Satsuma mandarin is in accordance with the favorable environmental conditions for occurrence of Sclerotinia twig blight, fungicide sprays at this period of time may reduce incidences Sclerotinia twig blight on trees and cottony rot on fruits.

Detailed and quantitative epidemiological data are essential for the development of effective and economical control programs for the diseases caused by *S. sclerotiorum* (Abawi and Grogan, 1979). Hockey (1959) reported that calyx-end rot on apple was incited by ascospores through fruits, leaves and petals during the flowering stage of about 3 weeks. Turkington and Morrall (1993) reported a significant correlationship between weather conditions and petal infections for occurrence of Sclerotinia twig blight on canola. Information on the source, nature, and availability of the primary inoculum and factors required for infection and soil indexing for viable sclerotia may allow the prediction of the timing of fungicide sprays for control of Sclerotinia twig blight on trees and cottony rot on fruits of citrus.

Use of resistant cultivars is one of the efficient management methods for plant diseases. In this experiment, some varieties of Satsuma mandarin such as Ichinan were relatively more resistant to Sclerotinia twig blight. Therefore, the varieties can be used as a source of breeding materials for resistance to *S. sclerotiorum* in the future.

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